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Thanks.

# Free and Glycosidically Bound Aroma Compounds in Pineapple (*Ananas comosus* L. Merr.)

Ping Wu, May-Chien Kuo, Thomas G. Hartman, Robert T. Rosen, and Chi-Tang Ho*

Department of Food Science and The Center for Advanced Food Technology, Cook College, New Jersey Agricultural Experiment Station, Rutgers, the State University of New Jersey, New Brunswick, New Jersey 08903

Free and glycosidically bound volatiles from pineapple juice were isolated and separated by means of an Amberlite XAD-2 column. Volatile compounds from bound fractions were released by almond β-glucosidase hydrolysis. By use of γ-valerolactone as internal standard, volatile components of free and bound fractions were determined by GC and GC–MS. Glycosidically bound 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), phenols, lactones, alcohols, acids, and aldehydes were observed in pineapple for the first time. Phosphatase was also used to hydrolyze the bound fraction, but no volatile compounds were released. Glycosidically bound DMHF was further confirmed by HPLC analysis.

## INTRODUCTION

In the past few years the analysis of flavor precursors and intermediates, especially glycosides in fruits such as grape, passion fruit, and papaya, has received increasing interest and attention (Engel and Tressl, 1983; Gunata et al., 1985; Heidlas et al., 1984; Strauss et al., 1986; Schwab and Schreier, 1988). These authors found that the aromatic components of fruits are present either in a free form or bound to sugar in the form of glycoside. Some conjugated forms of 2-phenylethanol, terpene alcohols, benzaldehyde, and others have been found. Our previous study (Wu et al., 1990) reported the presence of glycosidically bound 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) in pineapple. Glucoside of DMHF has recently been isolated and identified from strawberry juice (Mayerl et al., 1989). Meanwhile, Schwab and Schreier (1989) reported the monoterpene alcohols linalool and 2,6-dimethyloct-7-ene-2,3,6-triol were released by phosphatase activity in Carica papaya fruit. The present study reports the presence of glycosidically bound volatile compounds in pineapple by use of β-glucosidase to release aglycons.

## EXPERIMENTAL PROCEDURES

**Reagents.** The solvents (*n*-pentane, diethyl ether, methanol, and dichloromethane) were HPLC grade from Fisher Scientific Co. (Springfield, NJ). Diethyl ether, *n*-pentane, and dichloromethane were redistilled prior to use. The 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), δ-octalactone, γ-octalactone, γ-nonalactone, and γ-decalactone were obtained from Firmenich Inc. (Princeton, NJ). Standards of *n*-paraffins ($C_5$–$C_{26}$) were purchased from Alltech Associates (Deerfield, IL). Amberlite XAD-2 (20–60 mesh), acetic acid, ethyl acetate, 2-pentanol, hexanal, 2-butoxyethanol, γ-valerolactone, benzaldehyde, hexanoic acid, 4-hydroxybenzaldehyde, vanillin, eugenol, 4-allyl-2,6-dimethoxyphenol and (3-hydroxyphenyl)ethyl alcohol were obtained from Aldrich Chemical Co. (Milwaukee, WI). Almond β-glucosidase, acid phosphatase from sweet potato, glucono-δ-lactone, and EDTA were obtained from Sigma Chemical Co. (St. Louis, MO).

Fresh pineapples (*Ananas comosus* L. Merr.) grown in Costa Rica were purchased from a local market.

**Separation of Free and Bound Aroma Compounds.** The fresh clear juice was prepared from skinned, cut, cored, whole fruit (2.046 kg) by a high shear blender. About 1.1 L of clear pineapple juice was obtained by vacuum filtration through a bed of Celite 545 (J. T. Baker Chemical Co., Phillipsburg, NJ). The clear pineapple obtained was then passed through a solvent-washed (Gunata et al., 1985) Amberlite XAD-2 column [1 cm (i.d.) × 50 cm] with a flow rate of 2.0 mL/min. The column was rinshed with 100 mL of distilled water to eliminate sugar, acids, and other water-soluble compounds. The free volatile fraction of the aroma fixed on the column was eluted by using 1 L of pentane/ether (1/1) at a flow rate of 2.0 mL/min. The glycosidically and phosphate bound fraction was subsequently eluted by using 1 L of methanol. The methanol eluate was divided exactly into two parts and then concentrated to dryness by a stream of nitrogen. One portion of the dried material was dissolved in 100 mL of 0.2 M citric–phosphate buffer solution (pH 5). The buffered mixture was washed twice with 80 mL of pentane/ether (P/E) to remove possible existing traces of free volatiles. The P/E extracts were combined with the previously obtained free fraction eluate. The combined P/E was dried over anhydrous sodium sulfate and then concentrated to a final volume of 0.5 mL with nitrogen. The glycosidically bound compounds dissolved in the buffer solution were hydrolyzed by almond β-glucosidase (90 mg, 5.3 units/mg) at 37 °C for 72 h. In an analogous experiment, acid phosphatase from sweet potato (12.5 mg, 40 units/mg) was used for hydrolysis of the other portion of dried material. The phosphate buffer contained 0.2 M glucono-δ-lactone and 0.2 M EDTA to inhibit the glucosidase activity present in this enzyme preparation. The liberated aglycons were extracted with three 80-mL portions of dichloromethane. The extracts were dried over anhydrous sodium sulfate and concentrated to a final volume of 0.2 mL with a stream of nitrogen.

γ-Valerolactone (1.22 mg/mL of ether) was added as the internal standard to each fraction before concentration.

**GC and GC–MS Analyses of Volatile Compounds.** A Varian 3400 gas chromatograph equipped with a fused silica capillary column [50 M × 0.32 mm (i.d.); $d_f$ = 1.05-μm film thickness, DB-1; J & W Scientific] and FID was used to analyze the volatile components in each fraction. The operating conditions were as follows: injection temperature, 270 °C; detector temperature, 300 °C; helium carrier flow rate, 1.0 mL/min; temperature program, 40–260 °C at 2 °C/min and held at 260 °C for 40 min. A split ratio of 50:1 was used. Quantitative determinations were carried out by a Varian 4270 integrator. Linear retention indices were calculated against *n*-paraffins standards ($C_5$–$C_{26}$, Alltech Associates) as references (Majlat et al., 1974).

GC–MS analysis was accomplished by using a Varian 3400 gas chromatograph coupled directly to a Finnigan MAT 8230 high-resolution mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV and recorded on a Finnigan MAT SS 300 data system.

**Preparation of Samples for HPLC.** Pineapple clear juice was obtained by the same procedures described above. Five milliliters of each clear juice was passed thr ugh a Supelclean LC-18 (Supelco Inc., Bellef nte, PA) and a 0.45-μm filter (Lida Manufacturing Corp., Bensenville, IL).

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**High-Performance Liquid Chromatography.** Quantitative HPLC determinations were conducted with a Varian 5000 liquid chromatograph, a 2050 variable-wavelength detector, and a CDS 401 integrator. A reversed-phase, PartiSphere $C_{18}$ column [30 cm × 4.6 mm (i.d.)] (Whatman Inc., Clifton, NJ) was used. Chromatographic conditions were as follows: mobile phase A, 0.2 M acetate buffer, pH 4.06; mobile phase B, methanol, gradient (time, % A) (0, 100), (2, 100), (15, 60), (25, 60), (26, 100); flow rate, 1.0 mL/min; detector, UV 280 nm; and injection volume, 10 μL. The qualitative analysis of DMHF and its glucoside was conducted by use of commercial and synthesized (Mayerl, 1989) standards, respectively.

## RESULTS AND DISCUSSION

Table I shows the free and glycosidically bound volatile compounds observed in pineapple juice. The identification was accomplished by comparing GC retention indices and MS spectra with that of either authentic compounds or published data (Heller and Milne, 1980; Ten Noever de Brauw et al., 1983). The relative quantity of each compound was determined by using γ-valerolactone as the internal standard without considering recovery of volatiles and GC-FID response factors.

Most of the free volatile compounds shown in Table I were reported previously in pineapple (Van Straten et al., 1977; Takeoka et al., 1989). Among them, methyl 3-acetoxyhexanoate, DMHF, and methyl 5-acetoxyhexanoate were most abundant. Some alcohols such as 2-pentanol and 2-butoxyethanol, hexanoic acid, and phenols such as phenol, 4-hydroxybenzaldehyde, vanillin, and syringaldehyde were found for the first time in pineapple.

Recently, Schwab et al. (1989) reported the presence of phosphorylated terpenoid alcohols in papaya fruit. The bound fraction isolated from pineapple was therefore hydrolyzed by two types of hydrolases, β-glucosidase and acid phosphatase. Glucono-δ-lactone (0.2 M) and 0.2 M EDTA were added to inhibit the glycosidase activity present in the acid phosphatase. The free volatile fraction had "fruity", "pineapple-like" aroma, while the glycosidically or phosphate bound fractions had no odor. Only after enzymatic hydrolysis did the glycosidically bound fraction have the characteristic fruity, pineapple-like aroma. In contrast the β-glucosidase hydrolysis, the extract of phosphatase hydrolysate had no odor. No volatile compound was liberated by acid phosphatase as proven by GC and GC–MS.

The glycosidically bound volatiles isolated from pineapple were mainly hydroxy compounds and lactones. DMHF was the most abundant compound, followed by δ-octalactone and ethyl 3-hydroxyhexanoate. Some glycosidic hydroxy esters such as ethyl 3-hydroxyhexanoate and methyl 3-hydroxyoctanoate were found in pineapple for the first time.

It is interesting to note that many lactones such as γ-hexalactone, δ-hexalactone, δ-heptalactone, γ-octalactone, δ-octalactone, γ-nonalactone, and γ-decalactone were found in the glycosidically bound fraction. These compounds may be present in the form of glycosidically bound hydroxy acids in pineapple. Limonoid glucosides in citrus also exist as glucosidically bound δ-hydroxy acids (Hasegawa et al., 1989), although free limonoids are known as derivatives of triterpene δ-lactones. A hydroxy acid is both alcohol and acid. γ- and δ-hydroxy acids might lose water spontaneously to yield a lactone under acidic conditions. However, that lactonization happened during GC injection is not ruled out. Free γ-butyrolactone, γ-hexalactone, γ-octalactone, δ-octalactone, γ-nonalactone, γ-dodecalactone, and γ-hexadecalactone have been reported in pineapple (Van Straten, 1977; Takeoka et al., 1989).

**Tabl I. Free and Glycosidically Bound V latile Compounds of Pineapple**

| compd | RI (DB-1) | μg/kg (ppb) free | bound | ID |
|---|---|---|---|---|
| acetate acid | 579 | 109 | –[a] | b, c |
| ethyl acetate | 600 | 470 | – | b, c |
| 2-pentanone | 666 | 12 | – | b |
| ethyl propenoate | 678 | 10 | – | b |
| 2-pentanol | 682 | 7 | – | b, c |
| propyl acetate | 691 | 6 | – | b |
| methyl butanoate | 707 | 26 | – | b |
| 3-methylbutanol | 720 | 23 | – | b |
| 3-methylpentan-2-ol | 743 | 9 | – | b |
| methyl 2-methylbutanoate | 762 | 70 | – | b |
| hexanal | 778 | 10 | – | b, c |
| methyl pentanoate | 808 | 5 | – | b |
| 1-hexanol | 852 | – | 12 | b, c |
| 2-butoxyethanol | 889 | 74 | 24 | b, c |
| dimethyl malonate | 891 | 105 | – | b |
| γ-valerolactone | 908 | IS | IS | b, c |
| methyl 3-hexenoate | 914 | 5 | – | b |
| benzaldehyde | 936 | 11 | 9 | b, c |
| phenol | 956 | 54 | – | b |
| hexanoic acid | 959 | 23 | 11 | b, c |
| ethyl hexanoate | 982 | 37 | – | b |
| methyl 3-acetoxybutanoate | 1011 | 210 | – | b |
| γ-hexalactone | 1011 | – | 45 | b |
| methyl 3-hydroxyhexanoate | 1019 | 12 | – | b |
| 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone | 1031 | 700 | 491 | b, c |
| δ-hexalactone | 1049 | 26 | – | b |
| methyl 4-methylpentanoate | 1085 | 141 | – | b |
| 2-phenylethanol | 1090 | – | 19 | b, c |
| ethyl 3-hydroxyhexanoate | 1106 | 52 | 168 | b |
| methyl octanoate | 1116 | 34 | – | b |
| δ-heptanoate | 1147 | – | 34 | b |
| methyl 3-acetoxyhexanoate | 1176 | 1071 | – | b |
| methyl 4-acetoxyhexanoate | 1202 | 193 | – | b |
| methyl 5-acetoxyhexanoate | 1220 | 676 | – | b |
| γ-octalactone | 1221 | – | 8 | b, c |
| methyl 3-hydroxyoctanoate | 1243 | – | 29 | b |
| ethyl 3-acetoxyhexanoate | 1245 | 101 | – | b |
| δ-octalactone | 1250 | 99 | 226 | b, c |
| ethyl 4-acetoxyhexanoate | 1263 | 76 | – | b |
| ethyl 5-acetoxyhexanoate | 1302 | 52 | – | b |
| *p*-hydroxybenzaldehyde | 1317 | 45 | 6 | b |
| γ-nonalactone | 1330 | – | 6 | b, c |
| eugenol | 1338 | – | 18 | b, c |
| vanillin | 1365 | 23 | – | b, c |
| methyl 3-acetoxyoctanoate | 1365 | 116 | – | b |
| methyl 4-acetoxyoctanoate | 1377 | 6 | – | b |
| (3-hydroxyphenyl)ethyl alcohol | 1384 | – | 11 | b, c |
| methyl 5-acetoxyoctanoate | 1385 | 129 | – | b |
| cinnamic acid | 1390 | – | 65 | b |
| γ-decalactone | 1441 | – | 6 | b |
| ethyl 3-acetoxyoctanoate | 1441 | 13 | – | b, c |
| ethyl 4-acetoxyoctanoate | 1450 | 42 | – | b |
| 4-allyl-2,6-dimethoxyphenol | 1574 | – | 31 | b, c |
| syringaldehyde | 1617 | 80 | 27 | b |

[a] Not detected. [b] Mass spectral data from the following sources: Heller and Milne (1980), Ten Noever de Brauw et al. (1983). [c] GC and/or GC–MS analyses of authentic compounds.

Many glycosidically bound phenolic compounds such as eugenol, 4-hydroxybenzaldehyde, (3-hydroxyphenyl)-ethyl alcohol, 4-allyl-2,6-dimethoxyphenol, and syringaldehyde were also found in pineapple for the first time. Strauss et al. (1987) reported some conjugated forms of phenols such as propiovanillone, 2-(4-hydroxy-3-methoxyphenyl)ethanol, 4-(4-hydroxy-3-methoxyphenyl)-2-butanol, and dimethoxyphenol from grape juice. Van den Dries et al. (1989) reported the occurrence of relatively large amounts of glycosidically bound eugenol in the *Lamiaceae* species. The role of these compounds as precursors of phenolic flavorants is unknown.

Glycosidically bound alcohols, such as hexanol, 2-

butoxyethanol, (3-hydroxyphenyl)ethyl alcohol, and 2-phenylethanol, were also found in pineapple for the first time. Glycosidic hexanol and 2-phenylethanol have been found in papaya and apple (Schwab et al., 1988, 1989). Glycosidic 2-phenylethanol also has been found in grape, mango, and apricot (Salles et al., 1988).

In addition, glycosidic acids such as hexanoic acid and cinnamic acid have been found in the pineapple for the first time. Glycosidically bound geranic and neric acid have been found in the herb *Melissa officinalis* and ginger (van den Dries et al., 1989). Glycosidically bound 2-methylbutanoic acid/3-methylbutanoic acid, benzoic acid, and phenylacetic acid also have been found in papaya (Schwab et al., 1989). In addition to being found in papaya and grapes, glycosidically bound benzaldehyde was also observed in pineapple.

We previously reported the presence of glycosidically bound DMHF in pineaple (Wu et al., 1990). GC and GC–MS analyses of the glycosidically bound fraction showed DMHF as the most abundant compound. The results of HPLC analysis confirmed the presence of free DMHF and its glucoside in pineapple by comparing their retention times with that of the standards and by spiking the samples with authentic DMHF and its glucoside.

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Registry No. Acetic acid, 64-19-7; ethyl acetate, 141-78-6; 2-pentanone, 107-87-9; ethyl propenoate, 140-88-5; 2-pentanol, 6032-29-7; propyl acetate, 109-60-4; methyl butanoate, 623-42-7; 3-methylbutanol, 123-51-3; 3-methylpentan-2-ol, 565-60-6; methyl 2-methylbutanoate, 868-57-5; hexanal, 66-25-1; methyl pentanoate, 624-24-8; dimethyl malonate, 108-59-8; methyl 3-hexenoate, 2396-78-3; phenol, 108-95-2; ethyl hexanoate, 123-66-0; methyl 3-acetoxyhexanoate, 89422-42-4; methyl 3-hydroxyhexanoate, 21188-58-9; δ-hexalactone, 823-22-3; methyl 4-methylpentanoate, 2412-80-8; methyl octanoate, 111-11-5; methyl 3-acetoxyhexanoate, 21188-60-3; methyl 4-acetoxyhexanoate, 112059-09-3; methyl 5-acetoxyhexanoate, 35234-22-1; ethyl 3-acetoxyhexanoate, 21188-61-4; ethyl 4-acetoxyhexanoate, 121308-81-4; ethyl 5-acetoxyhexanoate, 35234-24-3; vanillin, 121-33-5; methyl 3-acetoxyoctanoate, 35234-21-0; methyl 4-acetoxyoctanoate, 60121-04-2; methyl 5-acetoxyoctanoate, 35234-23-2; ethyl 3-acetoxyoctanoate, 85554-66-1; ethyl 4-acetoxyoctanoate, 121312-01-4; 2-butoxyethanol, 111-76-2; benzaldehyde, 100-52-7; hexanoic acid, 142-62-1; 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 3658-77-3; ethyl 3-hydroxyhexanoate, 2305-25-1; δ-octalactone, 698-76-0; *p*-hydroxybenzaldehyde, 123-08-0; eugenol, 97-53-0.